

June 6, 2016

What We Did Today:

- Made Liquid LB Media
- Worked on Touch Tomorrow Posters
- Streaked Florescent Plates

LB Prep Protocol

Add 25g LB mix per Liter. Mix well, then autoclave for an hour and a half.

General Lab

Creation of LB media with 1x CAM antibiotic, creation of 50% Glycerol, creation of Deionized Water samples -MRG

Igem Preparation

Creation of LB agar plates with CAM antibiotic
Transformed GFP and RFP genes into *E.coli* cells -MRG

Ginny and Jingyi:

Tested Extractions with banana and strawberry

June 7, 2016

What we did today:

- Learned mammalian cell culture
- Thawed cells and plated
- Made Media
- Worked on poster and survey

Other team members:

- Made preps for strawberry extraction
- Built dark box

Spray hood down with ethanol, wipe from back to front, turn on UV light for a few minutes

Making media:

We made 100mL of media using: FBS (10% of final concentration), Glutamine (1% of final concentration), PS (1% of final concentration), and DMEM (88% of final concentration)
For us this time, that meant using 5 mL FBS, 0.5 mL Glut, 0.5 mL PS, and 44 mL DMEM

Thawing Cells Protocol:

5ml media then draw up the cell line into the same pipette. Slowly dispense into final tube. Mix lightly, let rest for a few minutes. Run through centrifuge at 800 RPM for 5 minutes
Pipette as much of it as you can off without disturbing the pellet of cells at the bottom of the tube. Dispose of old media in sterilized beaker
Flick the bottom of the tube to re-suspend the cells
Use approximately 6ml of media in the new flasks
Gently add and slowly pipette up and down in the tube. Pipette into the flask.
Label using ethanol resistant pen

General Lab

Creation of DMEM media containing 1% Penicillin Streptomycin, 10%FBS, and 1% Glutamine

Thawed HEK293T cells to create six cell lines -MRG

Creation of 100 mL cell culture media -JW

iGEM Preparation

Creation of Liquid media samples of GFP colonies containing 1x CAM antibiotic

Creation of Dark box for presentation of GFP and RFP expressing *E.coli* cells -MRG

Transformed cells with RFP, GFP

I13521 plate 3 well G6 TetR-RFP

10uL water to well

Thawed competent cells DH5alpha on ice

1uL DNA to 50uL cells heat shocked 45 seconds 42 degree water then back on ice

added 200uL liquid LB to cells

shaker 1.25 hours spread on plates -GM

DNA extraction trials with grapes and blueberries

Transformation (LacI RFP) -JW

June 8, 2016

What we did today:

- Split cells
- Made and plated agar
- Worked on survey/ printed poster
- Transformed cells

Other team members:

- Worked on bacterial plates
- Made the "traits" wall art

Made 1 L of agar:

- Mixed 40g LB agar with 1 L of water. Autoclave for an hour and a half, then let cool.
- Add 1 mL/Liter of CAM

Expand Cells from T25 Flask (Done in hood):

- Since most cells were happily growing, alive, and densely populated we decided to expand cells for reculturing instead of splitting them.
- Removed all media from T25
- 5 mL of PBS was put into T25 flask and swirled to make sure it covered all cells. Then completely removed
- 0.5 mL of trypsin was added to the cells in the T25 flask and swirled around to make sure the cells were completely coated.
- The flask was then knocked to unstick the cells from the flask.
- 5mL of media was then put into the flask with the cells and the trypsin and mixed by pipetting up and down vigorously.
- The media and cells were then moved from the T25 to the bigger flask.
- The cells were viewed in the new flask to make sure they were adequate and then stored in the incubator.

Transformation Protocol (Using InterLab Study):

- Thaw HD5(alpha) cells on ice
- 50 μ L cells/tube (5 tubes)
- Add 1 μ L DNA (spin before opening)
- Sit on ice for 30 mins
- Heat shock – 42°C water bath for 45 seconds
- Put back on ice
- 200 μ L LB liquid/tube
- On shaker platform for 1-2 hours
- Plate all to CAM plates
- Plates incubate at 37°C

*Changes to Protocol with interlab samples:

- Added 10 μ L diH₂O to samples with brown and light pink caps before putting with competent cells (could not get samples from tubes otherwise)

Splitting Cells

Checked incubated cells in flask under microscope. They were ~80% confluent and very densely grown in the middle of the plate. After sterilizing the hood with ethanol and UV light, we laid out: the flask with the cells,

Media (DMEM, Glutamine, PS, and FBS), PBS, and trypsin.

We first removed all of the old media, and disposed of it into the waste beaker. We then added 5 mL of PBS and gently swirled the flask before it was completely removed. .5 mL trypsin was added and swirled to cover the base of the plate. The flask was knocked while kept horizontal to unstick the cells from the base of the flask.

5mL of the media was added to the cells and trypsin, and vigorously pipetted up and down to break up the cell clumps. All of the liquid in the flask was then removed from the flask and added to a tube. 2 mL of the liquid in the tube was then added back to the flask. The remainder of the liquid in the tube was discarded in the waste beaker. The flask containing the media and the cells examined under a microscope to ensure the cells survived the splitting. After confirming the cells were successfully split, the flask was put back into the incubator.

Other tasks for the day:

Making agar

Plating agar into Pyrex dishes and square plates in preparation for Touch Tomorrow.

General Lab

Maintenance of HEK293T mammalian cell cultures

Cell culture split (1:3) -JW

Cells only about 30% confluent didn't split -GM

iGem Preparation

Creation of 1:10, 1:100, and 1:1000 dilutions of GFP *E.coli* in LB media and plated on CAM plates to test best dilution for creation of *E.coli* drawings

Creation of next generation of *E.coli* in LB media and 1x CAM

Creation of 3 RFP cultures in CAM, (2 with TetR-RFP and 1 with LACI-RFP)

June 9, 2016

What we did today:

Counted cells in well
Colored with bacteria

Split Cells
Checked on Transformation

What other team members did today:

Split cells

Counting cells protocol:

Check cells to see if they need to be split. If not, put them back in the incubator. If so, split first. Instead of throwing away the liquid (cells, media, trace amounts of trypsin) in the tube, keep the liquid to use for cell counting.

Pipette 10 μ L of the cells in media into microcentrifuge tube. Add 10 μ L of trypan blue to the microcentrifuge tube. Pipette up and down a few times to mix.

Take counting grid and cover, wipe down with ethanol. Place cover onto grid.

Take 10 μ L of the mixture of cells and trypan blue and pipette in the middle of the entrance to the grid cover. Ensure there are no bubbles and the mix spread out to cover the grid.

Check under microscope. Count all the cells in the outmost corner boxes, and the middle box of the grid

X		X
	X	
X		X

Take total, multiply by 4,000. This is your count of cells / mL

Take the concentration you want to have, then multiply by the number of wells you want to fill+1

Take the second number and divide by the first number. This is the mL of cells you will want to use.

Subtract the number of mL of cells from the number of mLs you want to end up with (usually the number of wells you want to fill+1). This is the amount of media you need to reach your final volume.

Mix these amounts into a centrifuge tube and mix by inversion. Pipette 1 mL into each well. Incubate.

Ex.

370 cells x 4,000 = 4,000 1.48 x 10⁶ cells / mL

500,000 x 7 = 3,500,000 in 7 mL

3.5 / 1.48 = 2.3649 mL of cells mixture needed

7 - 2.36 = 4.64 mL of media needed

384 7 wells

① Always

$$370 \times 4000 = 1.48 \times 10^6 \text{ cells per mL}$$

$$1 \times 10^5 \quad 500,000 \times 7 = 3.5 \times 10^6 \text{ in 7 mL}$$

$$3.5 \div 1.48 = 2.36 - \text{mL of cells}$$

$$7 - 2.36 = 4.64 \text{ mL of media}$$

$$1.5 \times 7 = 10.5 \text{ mL of cells}$$

$$10.5 \times 7 = 73.5 \text{ mL of media}$$

$$7 - 10.5 = -3.5 \text{ mL of media}$$

General Lab

Maintenance of HEK293T Cells

Creation of 1×10^5 , 3×10^5 , 5×10^5 cell plates for optimization testing

Cell count (Ginny's flask): 8×10^5 cells/mL

Cells plated at 4×10^5 cells/mL in 3 wells -JW

Split cells 1:4

Cell count 8×10^5 cells/mL, cells plated at 2×10^5 cells/mL in 6 wells - GM

iGem Preparation

Creation of fluorescent bacteria art for exhibition with 1:10 dilution liquid culture -JW

June 10, 2016

What we did today:

- Filled Isopropanol tubes
- Tested extractions
- Set up for TouchTomorrow
- Trimmed DNA Origami
- Put Up Remaining Posters
- Checked and Photographed Cell Plating Experiments
- Split Cells as Necessary

Sarah Cells: 40-50%

General Lab

- Observed of confluence of differential cell density optimization
- Maintenance of HEK293T cell cultures
- Cell culture split (1:4) -JW

iGem Preparation

Creation of fluorescent bacteria art for exhibition with 1:10 dilution liquid culture -JW